

only in one clone, it is difficult to distinguish between PCR or cloning artifacts and actual allelic variations without additional sampling. However, when the same base difference is found in more than one sequence it is unlikely to be from cloning errors. From the alignment of all 11 PBGD sequences a set of common bases emerged, the consensus or wild type allele sequence. Five of the eight clones (1.1, 1.3, 2.1, 3.3, and 5.3.) have the wild type amino acid sequence. Within this set with wild type amino acid sequence, there is only one difference at the nucleic acid level. At position 555, 4 of the 5 sequences have a dGTP while 1 along with the published erythropoietic and genomic PBGD have a dTTP. These appear to be two common alleles, which result in no amino acid difference. There are 2 base changes between clone number 1.1 and the published erythropoietic PBGD. An adenine to guanine change at base 513 (Leu 171) is a silent mutation, which is also present in 9 out of the 11 sequences, compared. The second difference is a cytosine to adenine substitution at base 995 (Thr 332.) This is not a silent change, with a threonine to asparagine non-conservative mutation. It appears however that the difference is an error in the published erythropoietic PBGD sequence since all 10 other sequences have an adenine at this position. In addition to these natural variations, there are three additional silent mutations introduced during the cloning at positions 1017, 1018 and 1020

B1
cont

to create a Mun-I site for future manipulations. The PBGD gene was ligated into pBluescript SK plasmid generating the pSK-PBGD 3988 bp plasmid, which was sequenced (see Fig. 1, Fig. 9a-9x and SEQ ID NO:9) --

Please replace Table 1 of page 23 as follows:

Table 1 Oligonucleotide primers:

Ico375-pbgds (32 mer) coding region 5' end w/ EcoRI site sense
5' CGT GGA ATT CAT GAG AGT GAT TCG CGT GGG TA 3' (SEQ ID NO:13)

Ico376-pbgda (47 mer) coding region 3' end w/ HindIII site antisense
5' GGA GAA GCT TAT TAA TGG GCA TCG TTC AAT TGC CGT GCA ACA TCC AG 3'
(SEQ ID NO:14)

Ico378-csnonc (20 mer) constitutive form non-coding sense
5' TCC AAG CGG AGC CAT GTC TG 3' (SEQ ID NO:15)

Ico379-esnonc (20 mer) erythropoietic form non-coding sense
5' TCG CCT CCC TCT AGT CTC TG 3' (SEQ ID NO:16)

Ico380-sinter (21 mer) internal coding sense
5' CAG CAG GAG TTC AGT GCC ATC 3' (SEQ ID NO:17)

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Ico381-ainter (21 mer) internal coding antisense
5' GAT GGC ACT GAA CTC CTG CTG 3' (SEQ ID NO:18)

Ico382-anonc (20 mer) non-coding sense
5' CAG CAA CCC AGG CAT CTG TG 3' (SEQ ID NO:19)

Ico383-pSKT7 (22 mer) pBluescript T7 promoter
5' GTA ATA CGA CTC ACT ATA GGG C 3' (SEQ ID NO:20)

Ico384- pSKpjrev (22 mer) pBluescript reverse1
5' CTA AAG GGA ACA AAA GCT GGA G 3' (SEQ ID NO:21)

Ico385- pSKrev (21 mer) pBluescript reverse2
5' CAG CTA TGA CCA TGA TTA CGC 3' (SEQ ID NO:22) --

Please replace the paragraph beginning at line 12 of page 29 with the following rewritten paragraph:

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--AATTCTAACA TAAGTTAAGG AGGAAAAAAA A ATG AGA GTT ATT CGT GTC GGT AC

(SEQ ID NO:23)

Met Arg Val Ile Arg Val Gly (SEQ
ID NO:24)

Plasmid pExp1 (Fig. 5) was made in a two step process.

Oligonucleotides ICO386 (5' AAT TCT AAC ATA AGT TAA GGA GGA
AAA AAA AAT GAG AGT TAT TCG TGT CGG TAC 3' (SEQ ID NO:25)) and
ICO387 (5' CGA CAC GAA TAA CTC TCA TTT TTT TTT CCT CCT TAA CTT
ATG TTA G 3' (SEQ ID NO:26)) were designed to provide upon
annealing a 5' *EcoR* I adhesive end and a 3' *Kpn* I sticky end.
Oligonucleotides ICO386 and ICO387 were annealed and ligated
with the *Kpn* I-*Hind* III PBGD fragment from pPBGD1.1 into *EcoR*
I -*Hind* III linearized pBluescript II SK- (Stratagene Cat #
212206) to yield plasmid pPBGD1.1Tra (Fig. 6). In the second
step the *EcoR* I -*Hind* III fragment from pPBGD1.1Tra was
ligated into pKK223-3 cut with the same enzymes resulting in
plasmid pExp1 (Fig. 5).

Please replace the paragraph beginning at line 12 of
page 30 with the following rewritten paragraph:

The following strategy was used. Plasmid pExp1
was cut with *Sal* I and *Bam*H I and the 5348 base-pair fragment
containing part of the tetracycline coding sequence and the
bulk of the plasmid was isolated. Into this was ligated the
Sal I-*Hind* III fragment from pBR322 (New England BioLabs Cat #

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303-3S, Lot # 50) containing rest of the coding sequence and an adapter formed by annealing oligonucleotides ICO424 (5' GATCACTCAT GTTTGACAGC TTATCATCGA TT 3' (SEQ ID NO:27)) and ICO425 (5' AGCTAATCGA TGATAAGCTG TCAAACATGA GT 3' (SEQ ID NO:28)). The adapter contains part of the tetracycline promoter and provides *Hind* III and *Bam*H I overhangs for ligation but destroys the *Hind* III and *Bam*H I restriction sites.

Please replace the paragraph beginning at line 24 of page 31 with the following rewritten paragraphs:

AATTCTAACA TAAGTTAAGG AGGAAAAAAAA A ATG AGA GTT ATT CGT GTC GGT AC
(SEQ ID NO:23)

Met Arg Val Ile Arg Val Gly

(SEQ ID NO:24)

B5

Oligonucleotides ICO386 (5' AAT TCT AAC ATA AGT TAA GGA GGA AAA AAA AAT GAG AGT TAT TCG TGT CGG TAC 3' (SEQ ID NO:25)) and ICO387 (5' CGA CAC GAA TAA CTC TCA TTT TTT TTT CCT CCT TAA CTT ATG TTA G 3' (SEQ ID NO:26)) were designed to provide upon annealing a 5' *Eco*R I adhesive end and a 3' *Kpn* I sticky end. Oligonucleotides ICO386 and ICO387 were annealed and ligated with the *Kpn* I-*Hind* III PBGD fragment from pPBGD1.1 into *Eco*R I -*Hind* III linearized pBluescript II SK- (Stratagene Cat # 212206) to yield plasmid pPBGD1.1Tra. In the second step the

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cont

EcoR I -*Hind* III fragment from pPBGD1.1Tra was ligated into pKK223-3 cut with the same enzymes resulting in plasmid pExp1.--

Please replace the two paragraphs beginning at line 3 of page 32 and ending at line 20 of page 32 with the following rewritten paragraphs:

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--Plasmid pExp1 was cut with *Sal* I and *Bam*H I and the 5348 base-pair fragment containing part of the tetracycline coding sequence and the bulk of the plasmid was isolated. Into this was ligated the *Sal* I-*Hind* III fragment from pBR322 (New England BioLabs Cat # 303-3S, Lot # 50) containing rest of the coding sequence and an adapter formed by annealing oligonucleotides ICO424 (5' GATCACTCAT GTTGGACAGC TTATCATCGA TT 3' (SEQ ID NO:27)) and ICO425 (5. AGCTAATCGA TGATAAGCTG TCAAACATGA GT 3' (SEQ ID NO:28)). The adapter contains part of the tetracycline promoter and provides *Hind* III and *Bam*H I overhangs for ligation but destroys the *Hind* III and *Bam*H I restriction sites. The resulting plasmid was called pExp1-M2.--

Plasmid pExp1-M2 was digested with *Pvu* I and *Afl* III and the larger of the two fragments corresponding to a size of 4745 base-pairs was isolated. This was ligated to the 1257 base-pairs long *Pvu* I-*Afl*III fragment derived from pUC19 containing the origin of replication and part of the

B ampicillin resistance gene to obtain plasmid pExp1-M2-Puc. This was passaged through JM110 and cut with BsaA1 and BsaB1 to excise the rom gene contained between the two sites and blunt-ended together to yield the final expression plasmid pExp1-M2-Puc-BB. The pExp1-M2-Puc-BB plasmid has been fully sequenced (see SEQ ID NO:11).

Please replace the paragraph beginning at line 8 of page 42 with the following rewritten paragraphs:

B ~~Met-Ser-Gly-Asn-Gly-Asn-Ala-Ala-Ala-Thr-Ala-Glu-Glu-Asn-Ser-Pro-Lys-Met-~~
Arg-Val... (SEQ ID NO:30)
ATG-TCT-GGT-AAC-GGC-ATT-GCG-GCT-GCA-ACG-GCG-GAA-GAA-AAC-AGC-CCA-AAG-ATG-
AGA-GTG.. (SEQ ID NO:29)

Please replace the paragraph beginning at line 24 of page 47 with the following rewritten paragraphs:

B ~~Normal Chromosomal Sequence:~~

5'-AG CGC ATG GGC TGG CAC AAC CGG GT-3' (SEQ ID NO:31)

Gln Arg Met Gly Trp His Asn Arg Val (SEQ ID NO:32)

AIP Chromosomal Sequence:

5'- AG CGC ATG GGC TAG CAC AAC CGG GT-3' (SEQ ID NO:33)

~~Stop-~~

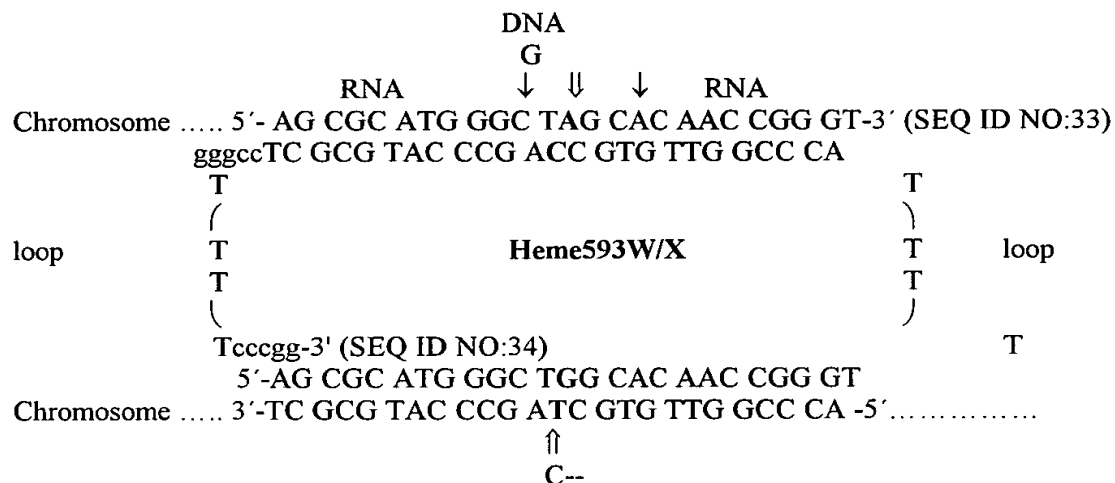
Please replace the paragraph beginning at line 1 of page 48 with the following rewritten paragraphs:

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~~SEP 17 2001~~

--The sequence of the chimeric oligonucleotide (Heme593W/X) is:

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IN THE SEQUENCE LISTING

Please substitute the attached Sequence Listing, numbered as pages 1-20 for the Sequence Listing previously submitted.